

**THROMBOLYTIC ACTIVITY OF CENTELLA ASIATICA LEAVES**

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**\*Corresponding authors e-mail:** [rishibd@gmail.com](mailto:rishibd@gmail.com)**ABSTRACT**

The methanol extract of *Centella asiatica* was estimated for thrombolytic effect. The extract demonstrated moderate thrombolytic activity which was 25.01 % where as the thrombolytic activity of standard was found 88.23 %. Thrombolytic indicates that have potent clot lysis property and anti-oxidative activity. The result showed momentous effect with comparison to standard thrombolytic agent, streptokinase. The obtained results support for the uses of this plant as traditional medicine.

**Keywords:** Thrombolytic Effect, Methanolic Extract and *Centella asiatica*.**INTRODUCTION:**

Many of the modern day's vital drugs and processed medicines are of plant origin. Medicinal plants contain different remedial agents which may have thrombolytic activity, antimicrobial activity, cytotoxic effect etc. Medicinal plants extract demonstrated that they can lyses thrombus as streptokinase<sup>[1]</sup>. Medicinal plants play a leading role in the treatment of varieties of human diseases from the dusk of human development<sup>[2]</sup>. *Centella asiatica* have been of medical interest due to their good therapeutic value in folk medicine<sup>[3]</sup>. Working with different medicinal plants extract showed that they can lyses thrombus as streptokinase<sup>[2, 4]</sup>. Thrombolysis is the breakdown (lysis) of blood clots<sup>[5]</sup> by pharmacological mean. It is colloquially referred to as clot busting for the reason. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator .The protein that normally activates plasmin. Thrombolysis requires the use of thrombolytic drugs, which are either derived from Streptokinase species. Some commonly used thrombolytics are: Streptokinase, Urokinase, Reteplase, Tenecteplase. Formation of blood clot lies at the basis of a number of serious diseases. By breaking down the clot, the diseases process can be arrested, or the complication reduced. While other anticoagulants (such as heparin)

decrease the growth of a clot, thrombolytic agent actively reduces the size of the clot. Diseases where thrombolysis is used: Myocardial infarction, Stroke (ischemic stroke)<sup>[6]</sup> Massive pulmonary embolism, Acute limb ischaemia.

**MATERIALS AND METHODS:****Collection and identification of the plant sample:**

The plant centella asiatica (Family: Umbelliferae) was collected from Dhaka and was taxonomically identified with the help of National Herbarium of Bangladesh, Dhaka. The leave and the stem were cut into small pieces and the sun dried for seven days. The leaves and stems were ground into coarse powder with the help of an attrition type of a grinder.

**Extraction of leaves:**

About 250 gm of powdered leaves was taken in a clean flat –bottomed glass container and percolated with 3 liters of Methanol. The container with its content was sealed and kept for 7 days with occasional shaking and stirring .the mixture was the filtered successively through a piece of clean white cotton .The filtrate thus obtained are kept in a open air for the evaporation of the methanol. After 10 to 15 days all the methanol are evaporated and I got the extract of methanol.

## THROMBOLYTIC EFFECT OF CENTELLA ASIATICA:

### Preparation of extract solution-

- 100 mg of extract is suspended in 10 ml of distilled water.
- Then it kept overnight.
- The soluble supernatant is decanted and filtered.

### Specimen preparation and In Vitro Thrombolytic Study:

5 micro centrifuge tubes are taken, sterilized, weighed. 6 ml blood is drawn from each volunteer. The blood is distributed in 5 different pre weighed (W<sub>1</sub>) micro centrifuge tube, each tube contain 0.5 ml of blood. The blood specimen is centrifuged at 2500 rpm for 5 minutes. Experiments for clot lysis were carried as reported earlier<sup>[7]</sup>. Then incubates the blood for 45 minutes 37° C. After clot formation i.e. incubation, the serum was completely removed by decantation, capillary absorption and by removing the serum from the inner surface of the tube. Kept the tubes at lying position on a tray for 6 minutes after first removal of serum and then remove the liquids of the tube surface by the cotton rod. Each tube was weighed (W<sub>2</sub>) again. Weight of clot is found as- Weight of clot = weight of clot containing tube (W<sub>2</sub>) – weight of tube alone (W<sub>1</sub>). Result varies for inappropriate weighing so it is done very carefully. Then I add 100µl of aqueous extract of centella asiatica plant extract to each micro centrifuge tube containing per-weighed clot. As a positive control, 100 µl of streptokinase is added to clot of standard tube. As a negative control, 100 µl of water is added to clot of blank tube. All the tubes are incubated at 37° c for 90 minutes and observed if clot lysis had occurred.

After 90 minutes of incubation, the released fluid is completely removed by decantation, capillary absorption and by removing the dissolved clot containing liquid from the inner surface of the tube carefully by cotton bar or by use of cotton tightly bound at top of a glass rod without disrupting the clot. The tubes are then weighed again. I have to ensure complete removal of released fluid, or the result will be erroneous. Keep the tubes at lying position on a tray for 6 minutes after first removal of released clot and then remove the liquids of the tube surface by the cotton rod. Weigh the tubes (W<sub>3</sub>) very carefully, please weigh very carefully, result varies for unsuitable weighing. The difference obtained in weight taken before and after clot lysis is expressed as percentage of clot lysis<sup>[7]</sup>.

$$\% \text{ of clot lysis} = (\text{wt of released clot /clot wt}) \times 100 \\ = (W_2 - W_3 / W_2 - W_1) \times 100$$

## RESULT AND DISCUSSION

The thrombolytic activity of standard was found 88.23% and for Crude extract, n-Hexane, and CCl<sub>4</sub>, respectively 24.52%, 26.53%, 24.00 % which indicates mild thrombolytic activity of Centella asiatica. Atherothrombotic diseases occur as serious impacts of the thrombus formed in blood vessels. Various thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels; but these drugs are not above limitations and can lead to serious and sometimes fatal consequences. Thrombolytic technique was used to examine the thrombolytic activity of Plant extracts in blood sample from healthy human volunteers, along with streptokinase as a positive control and water as a negative control<sup>[7]</sup>. SK, a known thrombolytic drug is used as a positive control<sup>[8]</sup>.

The comparison of positive control with negative clearly demonstrated that clot dissolution does not occur when water was added to the clot. On the basis of the result obtained in this present study we can say that the extract has moderate thrombolytic activity compared to negative control (water). However further research is necessary to find out the thrombolytic activity of the active compound.

One of the major causes of blood circulation problem is the formation of blood clots. Thrombi or emboli can lodge in a blood vessel and block the flow of blood in that location depriving tissues of normal blood flow and oxygen. This can result in damage, destruction (infarction), or even death of the tissues (necrosis) in that area. Fibrinolytic drugs has been used to dissolve thrombi in acutely occluded coronary arteries there by to restore blood supply to ischaemic myocardium, to limit necrosis and to improve prognosis<sup>[9]</sup>. Streptokinase is an antigenic thrombolytic agent used for the treatment of acute myocardial infarction. It reduces mortality as effectively as the nonantigenic alteplase in most infarct patients while having the advantages of being much less expensive. Tissue-type Plasminogen activator (tPA) is generally preferred as being effective and safer than either urokinase or streptokinase type activators. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency. Because of the shortcomings of the available thrombolytic

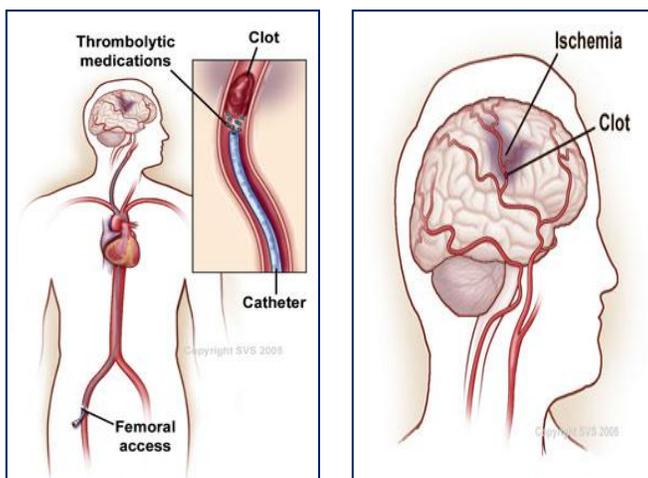
drugs, attempts are underway to develop improved recombinant variants of these drugs<sup>[10,11]</sup>.

### CONCLUSION:

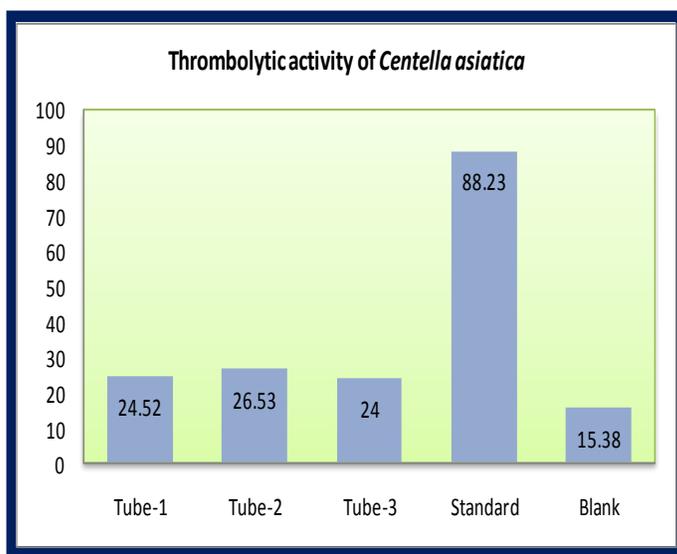
From this experiment, in summary, pharmacological evaluation of methanol extract of *Centella asiatica* has got the very good potential as a candidate for future thrombolytic. This is only a preliminary study and to make final comment the extract should methodically investigated phytochemically and pharmacologically to develop their medicinal and pharmaceutical potentialities.

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**Figure-1:** Thrombolytic Therapy



**Figure-2:** Graphical representation of Thrombolytic activity

**Table 1:** Data Analysis (Experiment)

Sample No.	W <sub>1</sub> (gm)	W <sub>2</sub> (gm)	W <sub>3</sub> (gm)	Sample No.	% of clot lysis
<b>Tube 1</b>	6.16	6.69	6.59	Tube-1	24.52
<b>Tube 2</b>	6.09	6.58	6.45	Tube-2	26.53
<b>Tube 3</b>	6.35	6.4	6.34	Tube-3	24
<b>Standard</b>	6.03	6.2	6.05	Standard	88.23
<b>Blank</b>	6.35	6.74	6.68	Blank	15.38

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